The product of the mammalian orthologue of the *Saccharomyces cerevisiae HBS1* gene is phylogenetically related to eukaryotic release factor 3 (eRF3) but does not carry eRF3-like activity

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Abstract We describe here the cloning and sequencing of human and mouse cDNAs encoding a putative GTP binding protein. Sequence comparison shows that these cDNAs (named eRFS) are likely to represent the orthologues of the yeast *Saccharomyces cerevisiae HBS1* gene and that the C-terminal domains of the encoded proteins share structural features with eukaryotic elongation factor eEF-1A and release factor 3 (eRF3) families. The phylogenetic analysis suggests that eRFS proteins and Hbs1p form a cluster of orthologous sequences branching with the eRF3 family. Nevertheless, in yeast, the human eRFS protein and Hbs1p do not complement eRF3/Sup35p thermosensitive mutation and do not interact with eRF1.

Key words: Translation; Phylogeny; *HBS1* gene; Eukaryotic release factor 3; Eukaryotic elongation factor 1A

1. Introduction

G proteins are a superfamily of GTP hydrolases involved in a wide variety of cellular mechanisms. Among them, translation elongation factors eEF-1A (also named EF-1α) and eEF-2, and release factor 3 (eRF3 in eukaryotes) interact transiently with the ribosome and catalyse GTP hydrolysis to aid in the progress of the translation process. eRF3, which shares high similarities with eEF-1A in its C-terminal domain, can be regarded as a paralogous group in eEF-1A phylogeny. In vitro, eRF3 binds to the release factor 1 (eRF1) and its GTPase activity stimulates eRF1 activity [1,2]. The C-terminal domain of eRF3 is required for yeast viability but the unique sequence at the N-terminus is non-essential [3]. Recently, two mouse genes, *GSPT1* and *GSPT2*, encoding proteins highly similar to human eRF3 have been sequenced [4]. Both mouse proteins directly interact with human eRF1 confirming the results obtained with eRF3 from yeast, human and *Xenopus laevis* [2,5,6].

Hbs1p, the translation product of the *Saccharomyces cerevisiae HBS1* gene, was classified as an eEF-1A-like protein because of its similarities with eEF-1A. Moreover, an increased copy number of *HBS1* suppressed the growth defect of a double mutant in *SSB1* and *SSB2* genes [7]. Ssb1/2p are molecular chaperones of the HSP70 family that are associated with translating ribosomes and may aid in the passage of the nascent polypeptide through the ribosome channel into the cytosol. Thus, it was suggested that Hbs1p has an eEF-1A-like activity, which is more efficient than the normal eEF-1A and could compensate for the less accessible aminoacyl-tRNA binding site in *ssb1ssb2* mutant strains [7].

In a recent search for chromosomal amplifications harbouring potential oncogenes in pancreatic cancer, CGH studies revealed an amplification on 6q23–24 [8]. Whereas the c-*myb* oncogene was identified as the most likely candidate oncogene in this amplification, a gene encoding a protein highly similar to the eRF3 family was found among the coamplified genes [9].

In this paper, we describe the cloning and sequencing of the human and mouse cDNAs encoding this eRF3-like protein that was named eRFS for 'RF similar'. Our phylogenetic studies show that *eRFS* gene is likely to correspond to the mammalian homologue of *S. cerevisiae HBS1* and reveal the relationship between the eEF-1A, eRF3 and eRFS families. We also show that *HBS1* and *eRFS* genes cannot complement yeast *eRF3/sup35(ts)* mutants and that Hbs1p does not interact with eRF1 or eRF3.

2. Materials and methods

2.1. Yeast strains and media

The *S. cerevisiae* strains 2-33G-D373 and 57-132-L28-2V-P3982 used for complementation experiments were previously described [2]. The *S. cerevisiae* strains SFY 526 [10] and HF7c [11] were used in two-hybrid assays. Yeast cultures were grown in YPD (1% yeast extract/2% peptone/2% glucose) or in SC synthetic minimal medium (0.67% yeast nitrogen base/2% glucose with appropriate auxotrophic supplements). Yeast transformations were performed according to Gietz et al. [12]. The plate colour assay with X-gal as substrate was essentially as described [13].

2.2. Plasmids

Unless indicated otherwise, DNA and RNA manipulations were carried out according to Sambrook et al. [14]. The multicopy (pSTR7) or centromeric (pUCH-U2) plasmids containing the complete yeast *SUP35* gene were described previously [15,16]. Plasmid pGADGH/SUP35 was constructed as follows: a 2-kb fragment of the *S. cerevisiae SUP35* sequence was amplified by polymerase chain reaction (PCR) using primers 26 (5′-CGCGGATCCGGATTCAAACCAAGGC-3′) and 16 (5′-CAAGACTCGAGCTCGGCAATTTTAAC-3′), digested with *Bam*HI and *Xho*I and inserted in *Bam*HI-*Xho*I sites of pGADGH. Plasmid pGBT9/SUP35 was constructing by subcloning a 2-kb *Eco*RI-*Xho*I fragment of the *S. cerevisiae SUP35*

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H.s. MARHRNVRGYNYDEDFEDDDLYGQSVEDDYCISPSTAAQFIYSRRDKPSVEPVEEYDYEDLKESSNSVSNHQLSGFDQAR 80

H.s. LYSCLDHMREVLGDAVPDEILIEAVLKNKFDVQKALSGVLEQDRVQSLKDKNEATVSTGKIAKGKPVDSQTSRSESEIVP 160
M.m.         MREVLGDAVPDDILTEAILKHKFDVQKALSVVLEQDGVQPWKEKSERAVCAGQPSKGKSVISRSSQSESEIVP 73
             ***********.** **.** *********.***** **  *.* * .* .*. .*** * *..*.*******

H.s. KVAKMTVSGKKQTMGFEVPGVSSEENGHSFHTPQKGPPIEDAIASSDVL-ETAS-KSANPPHTIQASEEQSSTPAPVKKS 238
M.m. KVAKMTVSGKKQTMGFEVPGLTSEENGLSVRAPHKGPPGDDVSVRSPNIPETGTPKSALPPPSLQTSEELGSTPTPVRKS 153
     ********************..***** *  .* **** .*    *  . **.* *** ** ..*.***  ***.**.**

                                    G1
H.s. GKLRQQIDVKAELEKR-QGGKQLLNLVVIGHVDAGKSTLMGHMLYLLGNINKRTMHKYEQESKKAGKASFAYAWVLDETG 317
M.m. GKLRQQIDVKAELENAVQGGKQLLNLVVIGHVDAGKSTLMGHMLYLLGNVNKRTMHKYEQESKKAGKASFAYAWVLDETG 233
     **************.  *********************************.****************************

        G2                  G3
H.s. EERERGVTMDVGMTKFETTTKVITLMDAPGHKDFIPNMITGAAQADVAVLVVDASRGEFEAGFETGGQTREHGLLVRSLG 397
M.m. EERERGVTMDVGMTKFETTTKVITLMDAPGHKDFIPNMITGAAQADVAVLVVDASRGEFEAGFETGGQTREHGLLVRSLG 313
     ********************************************************************************

        G4
H.s. VTQLAVAVNKMDQVNWQQERFQEITGKLGHFLKQAGFKESDVGFIPTSGLSGENLITRSQSSELTKWYKGLCLLEQIDSF 477
M.m. VTQLAVAVNKMDQVNWQQERFQEITGKLGHFLKQAGFKESDVAFIPTSGLSGENLTARSQSSDLTTWYKGMCLLEQIDSF 393
     ****************************************.************ .*****.** ****.*********

H.s. KPPQRSIDKPFRLCVSDVFKDQGSGFCITGKIEAGYIQTGDRLLAMPPNETCTVKGITLHDEPVDWAAAGDHVSLTLVGM 557
M.m. KPPQRSIDKPFRLCVSDVFKDQGSGFCVTGKIEAGYIQTGDRLLAMPPNETCTAKGITLHDEPVDWAAAGDHVNLTLVGM 473
     ***************************.************************.****************** ******

H.s. DIIKINVGCIFCGPKVPIKACTRFRARILIFNIEIPITKGFPVLLHYQTVSEPAVIKRLISVLNKSTGEVTKKKPKFLTK 637
M.m. DIIKINVGCIFCGPKEPIKACTRFRARILVFNIEVPITKGFPVLLHYQTVSEPAVIKRLISVLNKSTGEVTKKKPKLLTK 553
     *************** *************.****.****************************************** ***

H.s. GQNALVELQTQRPIALELYKDFKELGRFMLRYGGSTIAAGVVTEIKE 684
M.m. GQNALVELQTQRPVALELYKDFKELGRFMLRYGGSTVAAGVVTEIKE 600
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Fig. 1. Alignment of the predicted amino acid sequences of human and mouse eRFS. Conserved GTP binding motifs G1–G4 [25] are indicated by solid lines, identical amino acids by asterisks and similar amino acids by dots.

sequence generated by PCR using primers 57 (5′-GCAGAATTCTCGGATTCAAAC-3′) and 16 (5′-CAAGACTCGAGCTCGGCAATTTTAAC-3′) in the *Eco*RI-*Sal*I sites of plasmid pGBT9. Plasmid pGADGH/SUP45 was constructed by subcloning a 1.3-kb *Bam*HI-*Xho*I fragment of the *S. cerevisiae SUP45* sequence generated by PCR using primers 56 (5′-CGCGGATCCGGATAACGAG-3′) and 64 (5′-GATCCCTCGAGTTCGTCATAATATTC-3′) in the *Bam*HI-*Xho*I sites of plasmid pGADGH. Plasmid pGBT9/HBS1 was constructing by subcloning a 1.8-kb *Bam*HI-*Sal*I fragment of the *S. cerevisiae HBS1* sequence generated by PCR using primers 76-*Bam*HI (5′-TCGAGA*GGATCC*ACAGTGACTAC-3′) and 77-*Sal*I (5′-CATCTATTTTC*GTCGAC*CTACTGAG-3′) in the *Bam*HI-*Sal*I sites of pGBT9. Plasmid pFL38/HBS1 was constructed by inserting a 3-kb fragment containing the *HBS1* promoter and coding sequence in pFL38 centromeric vector [17]. Plasmid pFL44/hu-eRFS was constructed as follows: plasmid pFL44S [17] was digested by *Eco*RI and *Bam*HI and ligated with the 0.7-kb *Eco*RI-*Bam*HI fragment of pRS426/XSUP35 [2] containing the yeast *SUP35* promoter. The resulting plasmid was cut with *Bam*HI and *Xba*I and ligated with the 2.4-kb *Bam*HI-*Xba*I fragment from pSPORT/hu-eRFS which contained the full-length cDNA of the human eRFS gene. Plasmid pYX242/hu-eRFS was constructed by subcloning the 2.4-kb *Bam*HI-*Xba*I fragment of pSPORT/hu-eRFS in the *Bam*HI-*Nhe*I sites of pYX242 (R&D Systems).

2.3. Selection and sequencing of human and mouse eRFS cDNAs

The human *eRFS* gene was isolated by chance in a search for genes harbouring the chromosomal area 6q24 [9]. Exon trapping was performed with genomic DNA from the 620-kb Yeast Artificial Chromosome ICRFy900A0311Q according to the detailed protocol of Church et al. [18]. One of the exon trap fragments encoding a sequence similar to eEF-1A was used as a probe for the screening of an oligo(dT)-primed pancreas cDNA library at stringent conditions. Only one positive cDNA clone with an insert size of 1927 bp was found and sequenced on both strands. As the isolated cDNA clone did not contain the full-length sequence, the 5′ end was cloned with the 5′ RACE system (Gibco BRL). The resulting 2508-bp cDNA sequence (GenBank accession number U87791) was named human *eRFS*.

The mouse *eRFS* cDNA was selected fortuitously during the screening of a 10-day mouse embryo cDNA library (Novagen) with the 2-kb *Apa*I fragment of human GSPT1 cDNA encoding eRF3 [19]. Four positive clones were revealed in non-stringent conditions and the inserts were sequenced on both strands using standard methods [20]. Two of the cDNAs with almost perfect identity with human GSPT1 cDNA likely correspond to the mouse homologue of the GSPT1 gene. Both of the remaining cDNAs were identical and shared lower homologies with GSPT1 cDNA. The 5′ end of the cDNA was obtained by the 5′ RACE method using the RACE kit from Boehringer and three independent preparations of total mouse RNA. The resulting 2663-bp cDNA (GenBank accession number AF087672) was named mouse *eRFS*.

2.4. Northern blot

Northern blot membranes containing 2 μg of poly(A)$^+$ mRNAs from different human tissues or from mouse embryos at various stages (Clontech) were probed as described [14] with radiolabelled DNA fragments of the same origin. The membranes were washed at stringent conditions and *eRFS* mRNA bands were revealed by autoradiography.

2.5. Computer-assisted sequence comparisons and phylogenetic analyses

Alignment of the sequences was carried out visually with the help of the ED program of the MUST package version 1.0 [21]. Phylogenetic trees were constructed with maximum likelihood (ML), maximum parsimony (MP) and distance-based methods (neighbour joining, NJ) with the programs PROTML [22] version 2.3, PAUP version 3.1 and NJ in the MUST package version 1.0 [21], respectively. The

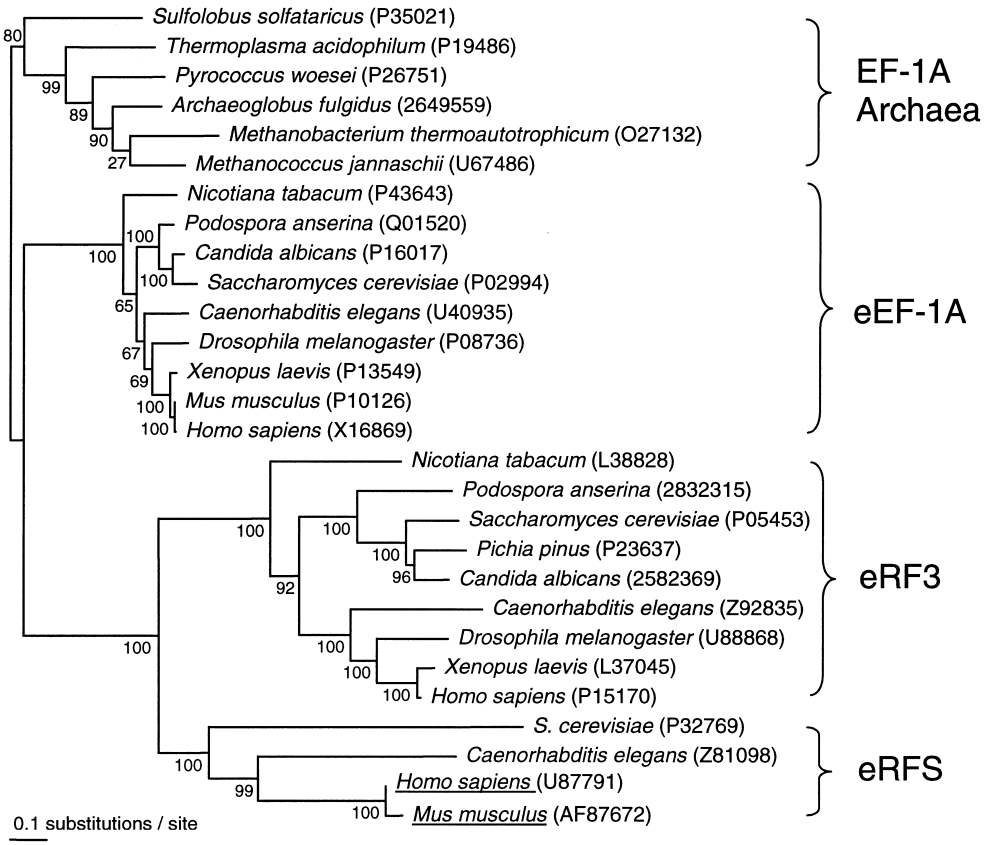

Fig. 2. Phylogenetic tree based on comparison of major eEF-1A, eRF3, and eRFS sequences from Eukaryotes. The outgroup consists of diverse EF-1A sequences from Archaea. The tree was constructed with the maximum likelihood method. Bootstrap proportions are shown below the corresponding nodes. For each sequence data base access numbers are indicated in parentheses. The scale bar indicates the numbers of substitutions per site for a unit branch length.

distances were computed with the substitution model of Kimura [23]. MP trees were obtained by 100 random addition heuristic search replicates and ML trees by the quick add OTUs search, with the JTT model of amino acid substitution and retaining the 5000 top ranking trees (options -jf -q -n 5000). Bootstrap proportions were calculated by the analysis of 1000 replicates for MP and NJ analyses. For ML analysis, bootstrap proportions were computed using the RELL method [24] because of computing time limitations.

3. Results

3.1. Sequence analysis of human and mouse cDNAs homologous to yeast HBS1 gene and structure of the predicted proteins

The human *eRFS* cDNA was isolated in a screen originally intended to find new genes from the chromosomal region 6q24 which shows chromosomal aberrations, e.g. high copy amplifications and heterozygous deletions, in pancreatic cancer [9]. The mouse *eRFS* cDNA was isolated from a 10-day mouse embryo cDNA library during the search for mouse *eRF3* cDNA.

The nucleotide sequence of human *eRFS* cDNA revealed a long open reading frame (ORF) encoding a polypeptide of 684 amino acids (Fig. 1) with a predicted molecular mass of approximately 75 kDa. The protein contains two domains: a 253 ami-o acid N-terminal part, showing no homology to any known protein and a 431-amino acid C-terminal domain with characteristic consensus GTP binding sites [25].

The mouse *eRFS* cDNA contains a single ORF encoding a polypeptide of 600 amino acids having 89% identity with the human polypeptide (Fig. 1). The major differences were found in the N-terminal part of mouse polypeptide, which was 87 amino acids shorter than human eRFS and shared only 70% identity with the remaining human eRFS N-terminal portion. This divergence was confirmed by three independent RACE experiments. The C-terminus harbouring the GTPase consensus motifs was 97% identical to the human polypeptide C-terminal domain.

3.2. Sequence comparison and phylogenetic analysis of eRFS and Hbs1p

The protein sequences homologous to eRFS were identified by a BLAST search using the eRFS sequence from mouse as the query sequence. This search revealed a high similarity of mouse eRFS with Hbs1p from *S. cerevisiae* and with a sequence from *Caenorhabditis elegans*. Because Hbs1p was classified first as an eEF-1A-like protein but also because it was noted that Hbs1p shares high similarity with eRF3 [7], sequences of the eRFS family were compared with those of the eEF-1A and eRF3 families. With the exception of the non-conserved N-terminal domain in eRFS and eRF3 families, the C-terminal domain of the predicted eRFS proteins can be unambiguously aligned with the archaebacterial EF-1A and the eukaryotic eEF-1A and eRF3 over 401 amino acid positions. The highest similarities were found in the region of the conserved GTP binding motifs G1–G4 shown for eRFS in Fig. 1.

The phylogenetic trees inferred with MP, NJ and ML methods were very similar and only the ML tree is shown (Fig. 2). The archaebacterial EF-1A was used as an outgroup and the root was thus located at the base of the Archaea. The close relationship of eukaryotic eEF-1A, eRF3 and eRFS was reinforced by the sharing of a small insertion, located at position 121–127 of human eEF-1A, which is only present in crenotes [26]. The major feature of the tree is the existence of four monophyletic groups, strongly supported by statistical analysis (bootstrap values close to 100%): the archaebacterial EF-1A, the eukaryotic eEF-1A, the eukaryotic eRF3 and the eukaryotic eRFS.

Our analysis shows that eukaryotic eRF3 and eRFS are sister groups, suggesting that an initial duplication created the eEF-1A encoding gene and an ancestral *RF* gene, which underwent another duplication, leading to the *eRF3* and *eRFS* genes. In fact, the evolutionary rates of the different paralogous genes are quite different, as evidenced by the difference in branch lengths (Fig. 2). A comparison of the distances between the three species simultaneously available for the three proteins (*Saccharomyces*, *Caenorhabditis* and *Homo*) revealed that eRF3 and eRFS evolved about 2.9 and 4.9 times faster than eEF-1A, respectively.

3.3. *HBS1 and human eRFS gene cannot complement yeast sup35 thermosensitive mutants*

We next addressed the question of whether the eRFS protein family is involved in translation termination. For this purpose, we tested the ability of human eRFS and Hbs1p to restore growth at 37°C and to complement the suppressor phenotype of *eRF3/sup35(ts)* mutants.

Yeast strain 2-33G-D373 containing the *eRF3/sup35(ts)* mutation was transformed with multicopy or centromeric plasmids carrying either the yeast *HBS1* gene or the human *eRFS* gene for complementation assays. The yeast *eRF3/SUP35* gene expressed from the same vectors served as control. For each plasmid, five independent transformants were picked at the permissive temperature (25°C) and analysed at the restrictive temperature (37°C). As shown in Fig. 3, none of the plasmids carrying either the *S. cerevisiae HBS1* gene (Fig. 3A) or the human *eRFS* gene (Fig. 3B) was able to compensate the growth defect of *eRF3/sup35(ts)* mutants at 37°C. These results suggest that the proteins encoded by the *eRFS* family cannot act as eRF3 in the translation termination complex.

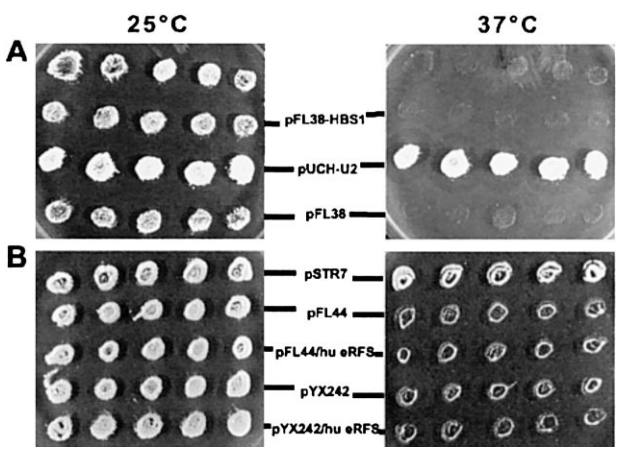

Fig. 3. Complementation of the yeast *eRF3/sup35(ts)* mutation by the *S. cerevisiae HBS1* gene (A) or the human *eRFS* gene (B). The *S. cerevisiae* temperature-sensitive strain 2-33G-D373 was transformed by the plasmids indicated. Five Leu^+ or Ura^+ transformants were replica-plated on YPD plates and incubated at 25°C or 37°C.

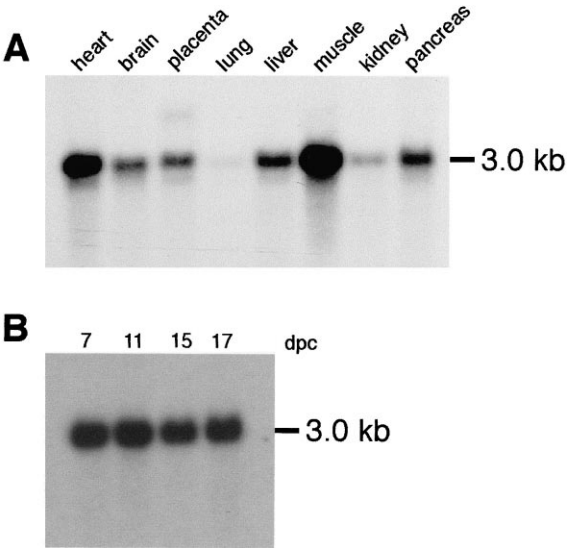

Fig. 4. Northern blot analysis of poly(A)-tailed mRNA from different human tissues probed with human *eRFS* cDNA (A) and from mouse embryo at different days post coitum (dpc) probed with mouse *eRFS* cDNA (B).

To go further, we studied the growth potential of transformants overexpressing Hbs1p in the 57-132-L28-2V-P3982 yeast strain carrying various nonsense alleles which confer different types of auxotrophy. Whichever the nonsense mutation tested, *ade1-14*, *his7-1* or *lys2-89*, Hbs1p did not influence the efficiency of suppression caused by the *sup35-57* mutation (data not shown). This result indicates that Hbs1p overexpression does not produce an antisuppressor phenotype and cannot compete with the suppressor effect of Sup35p mutations, and thus strongly suggests that Hbs1p does not contain eRF3-like release factor activity.

3.4. *Hbs1p does not interact with the components of the translation termination complex*

The GAL4-based two-hybrid system [27,28] was employed to test the interaction of Hbs1p with *S. cerevisiae* eRF3/Sup35p and eRF1/Sup45p. The plasmid carrying the Hbs1p-Gal4 fusion protein was transformed in different pair-wise combinations with eRF3/Sup35p-Gal4 or eRF1/Sup45p-Gal4 fusion proteins into yeast recipient strains carrying either *LacZ* or *HIS3* reporter genes (Table 1). The activation of the *GAL1* promoter was assayed by colony colour using the chromogenic substrate X-gal (*LacZ* reporter) and by growth on selective medium lacking histidine (*HIS3* reporter). The interaction between yeast eRF3/Sup35p and eRF1/Sup45p reported previously [5] served as a control. The results showed that neither eRF1/Sup45p nor Hbs1p fusion proteins expressed alone, nor double transformants expressing Hbs1p fusion protein together with eRF1/Sup45p or eRF3/Sup35p fusion protein activated reporter genes (Table 1). These results indicate that Hbs1p does not interact with eRF3 or eRF1.

Table 1
Interactions of Hbs1p with Sup35p and Sup45p measured in two-hybrid assays

pGADH insert	pGBT9 insert	Strain HF7C		Strain SFY526
		Colony color	Growth on SC-His	Colony color
SUP45	*SUP35*	Blue	+	Blue
HBS1	*SUP35*	White	−	White
HBS1	*SUP45*	White	−	White
None	*SUP45*	White	−	White
None	*HBS1*	White	−	White

3.5. Expression of human and mouse eRFS mRNAs in various tissues

The pattern of *eRFS* poly(A)-tailed mRNA was examined in various human tissues by Northern blot hybridisation using human *eRFS* cDNA as a probe. A 3.0-kb transcript was detected in all tissues examined, though transcript levels were of low abundance in lung and kidney and highly abundant in heart and skeletal muscles (Fig. 4A). Analysis of mouse embryo mRNAs with a mouse *eRFS* cDNA probe showed that the *eRFS* gene is strongly expressed at least during the late stages of mouse embryo development (Fig. 4B).

4. Discussion

In this study, we present the sequence of human and mouse cDNAs, of which the putative encoded polypeptides bear the conserved motifs of the GTP binding protein family. Phylogenetic analysis reveals that these polypeptides, named eRFS, are likely to correspond to the mammalian homologues of the translation product of the *S. cerevisiae HBS1* gene. The function of Hbs1p is not yet established. It has been reported that Hbs1p may be involved in translation elongation and speculated that Hbs1p could act as eEF-1A and bring the aminoacyl-tRNA to the ribosome [7]. The resemblance of Hbs1p to eRF3 was also mentioned [29].

Our phylogenetic study shows that the mammalian eRFS together with a protein of *C. elegans* are the orthologues of Hbs1p, which form a monophyletic group. This analysis indicates that three paralogous genes exist in eukaryotes, encoding eEF-1A, eRF3 and eRFS, and suggests a sister group relationship between eRF3 and eRFS. The presence of a variable domain at the N-terminus also distinguishes eRF3 and eRFS from eEF-1A. However, the precise relationship between eRF3 and eRFS is difficult to infer because the branches of eRF3 and eRFS are much longer than that of eEF-1A and might be grouped together only because of a tree reconstruction artefact known as the long branch attraction phenomenon [30]. The clear differences in evolutionary rate demonstrate that the functional constraints are quite different for the three proteins. As expected, eEF-1A is the most constrained, because of its central role in translation and its interaction with many other proteins. The evolutionary rate of eRFS is the highest suggesting limited functional constraints.

The function of eRFS was then evaluated on the basis of the phylogenetic study. Genetic analysis using *eRF3/sup35(ts)* mutants and the two-hybrid system clearly shows that neither human eRFS nor Hbs1p can replace eRF3 in the translation termination complex or interact with the components of this complex, i.e. eRF1 or eRF3. Moreover, in an in vitro assay [31], mouse eRFS did not exhibit GTPase activity in the presence of ribosomes and human eRF1 (L. Frolova, personal communication). Together, these results strongly suggest that the eRFS protein family is not directly involved in the translation termination process.

One can speculate about the function of eRFS in translation. A role of Hbs1p in translation elongation remains possible. However, a role in another cellular mechanism must be considered. Recently, it was demonstrated that an evolutionarily conserved GTP binding protein, named U5-116kD, closely related to the ribosomal elongation factor eEF-2, is a component of U5 snRNP which is essential for pre-mRNA splicing [32]. Like eRF3 and eRFS, U5-116kD protein contains an N-terminal domain which is absent in eEF-2. Nevertheless, the presence of a variable N-terminal domain cannot account for the functional differences of paralogous GTP binding proteins. Indeed, it was demonstrated in vitro that the C-terminal part of *X. laevis* eRF3 carries the GTPase activity required for eRF1 stimulating activity [1,2,6]. In yeast, the expression of the eRF3/Sup35p C-terminal part alone generates an antisuppressor phenotype [3]. Thus, despite extensive structural homologies in the conserved C-terminal domain, the paralogous proteins, eEF-1A, eRF3 and eRFS, may have non-overlapping functions and their close phylogenetic relationship does not allow a conclusion regarding their contribution to the same cellular process. Clearly, further experimentation such as the disruption of the *HBS1* gene in yeast is required to elucidate the function of the eRFS family.

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